that underwent cold treatment at 8 weeks post-inoculation were separated into two groups; one inoculated in June, and one inoculated in late August to evaluate the effect of accelerated symptom development due to hot mid-summer temperatures. All vines were tested with qPCR prior to cold treatment to determine infection status. Following 8 weeks of cold treatment, all vines were grown back from dormancy for 20 weeks and evaluated for reoccurrence of disease by symptom observation and qPCR testing. In this study, cultivar susceptibility and higher temperatures during initial infection stages had a greater impact on disease reoccurrence than duration of infection prior to cold treatment. This suggests that use of tolerant or resistant plant material should be a priority for areas at risk of Xf infection, and that summer temperatures need to be considered in addition to winter temperature and time of inoculation, when gauging likelihood of vine recovery. Further information regarding the effect of climate factors such as temperature on pathogen persistence is important to inform region-specific management strategies, and to evaluate the risk of Xf spread in new areas.

Phenotypic characterisation of two Spanish strains of *Xylella fastidiosa* subsp. *multiplex* ST6 differing in plasmid content

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Abstract: Two *Xylella fastidiosa* (Xf) subsp. *multiplex* strains IVIA5901 and ESVL were isolated from symptomatic almond trees in Alicante (Spain). Even if these Xf strains show an average nucleotide identity at the chromosomal level of 99.99%, they differ by the presence of two plasmids pXF64-Hb_ESVL and pUCLA-ESVL, only found in strain ESVL. Xf colonisation and disease development in host plants have been shown to be related to the size of cell aggregates, bacterial motility and biofilm formation, which are mediated by type I and type IV pili, among other traits. The goal of this study was to characterise the phenotypic characteristics of these strains that may be related to their differences in plasmid content.

The Spanish Xf subsp. *multiplex* strains were compared with Xf subsp. *multiplex* strains Alma-Em3 and BB08-1 isolated from blueberry and the reference strain Xf subsp. *fastidiosa* Temecula1 from grapes, all isolated in the US. To study bacterial behaviour and phenotypic characteristics, several experiments were performed to determine adhesion force to substrate, biofilm formation, movement, cell–cell aggregation, twitching motility and patterns of bacterial growth. Additionally, a virulence assays were conducted in the greenhouse using tobacco plants cvs. SR1 and Xanthi. We also determined the presence of genes coding for type I and type IV pili in the Spanish strains. Our results show that the two Spanish isolates of Xf subsp. *multiplex* have lower motility, less capacity of aggregation, make less biofilm, and cause lower disease severity when compared with the US isolates used in this study. Also, there were significant differences in disease severity between Xf Spanish strains that varied according to the tobacco cultivar, with Xanthi being the most susceptible one. Besides, our results indicate that strain ESVL exhibits stronger attachment to substrate than IVIA5901, but no differences in biofilm formation, cell–cell aggregation or twitching motility were found between them. The role of plasmids in the biology of these Spanish Xf strains will be further investigated.

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Ancestral state reconstructions of *Xylella fastidiosa*–host plant relationships

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